(Check) 11 - abnormality_analysis_of_oil_palm

by Arta Moro Sundjaja

FILE	11_-_ABNORMALITY_ANALYSIS_OF_OIL_PALM.PDF (337.69K)		
TIME SUBMITTED	17-APR-2017 02:59PM	WORD COUNT	3411
SUBMISSION ID	649644456	CHARACTER COUNT	18580

ABNORMALITY ANALYSIS OF OIL PALM (ELAEIS GUINEENSIS JACG) SOMATIC EMBRYO USING RANDOMLY AMPLIFIED POLYMORPHISM DNA (RAPD) AND RANDOMLY AMPLIFIED DNA FINGERPRINTING (RAF) TECHNIQUES

NESTI F. SIANIPAR[1], GUSTAAF A. WATTIMENA[2], MAGGY THENAWIJAYA S[3]. HAJRIAL ASWIDINNOOR[2], NURITA TORUAN-MATHIUS[4], GALE GINTING[5]

[1]Department of Biology, Faculty of Science and Mathematics, Universitas Pelita Harapan, Tangerang
[2]Department of Agronomy, Bogor Agricultural University, Bogor
[3]Department of Food Science and Technology, Bogor Agricultural University, Bogor[4]SEAMEO Biotrop, Bogor
[5]Oil PalmResearchCenter of Indonesia, Medan

ABSTRACT

Abnormality of somatic embryo (SE) can be resulted from genetic or epigenetic changes. Thus, genetic changes can be analyzed by *Random Amplified Polymorphism DNA*(RAPD) and *Randomly Amplified DNA Fingerprinting (*RAF*)* techniques. The objective of this research was to study the genetic changes of SE at several growth stages and their plantlets using RAPD and RAF. Through RAPD technique the SE normal and abnormal cotyledon could be distinguished using five primers (OPE-14,OPC-9, W-15, AP-20 and SC10-19) at clone 638, while the SE normal and abnormal cotyledon at clone 558 could be distinguished using three primers (OPE-14, W-15 and AP-20).Among these five primers pre-mentioned above, three primers, such as OPE-14, W-15 and AP-20 were differentin the SE normal and abnormal cotyledon at clone 638, at a specific band of 1750 bp. Furthermore, the RAF technique detected the changes of DNA genome at 90 – 358 bp. There were three amongstsix primers (AO-12, BB-18, W-15) genomic DNA sequences were detected at 150 bp. Moreover, the primer AB16 could detect the sequence DNA change of SE normal and abnormal cotyledon until one base pair at 95 bp. Consequently, RAPD and RAF technique with the primers W 15 and AP 20 could distinguish normal and abnormal SE cotyledon, plantlet and normal mother plant.

Key words: *somatic-embryoabnormality, oil-palm, RAPD, RandomlyAmplified DNA Fingerprinting (RAF).*

INTRODUCTION

Oil Palm (*Elaeis guineensis*) is a kind of cross breeding plants which its offspring produced heterozygous. However, derived plants of oil palm can be produced homozygous by such technique of tissue culture. There is a series of advantages of tissue culture method, such as produceshigh similarity between the derivatives and

mother plant in a period of short time and in a large amount. Propagation of oil palm seedlings through tissue culture can be done through the somatic embryo formation. However, some of the resulted derivativessometimes show a certain high somaclonal variation which can be investigated through an abnormality in cytology, mutation genotype, karyotype changes and changes in DNA sequence(Duncan 1997; Kaeppler *et al*., 2000).In addition to that, according to Deambrogio & Dale (1980) somaclonal variation can be inducedby addition of a high concentration of 2,4 D during the process.

Somaclonal variability is the genetic diversity of plants produced during tissue culture (Larkin & Scowcroft 1981).Genetic diversity occurs in tissue culture is caused by doubling the number of chromosomes (fusion, endomitosis), chromosome structure changes, gene changes and cytoplasm changes (Griffith et al. 1993; Kumar 1995). Furthermore, according to van Harten (1998), somaclonal variation may be caused by mitotic irregular, the role of chromosome instability and gene deletion.

There are several opinions about the occurrence of a genetic abnormality (Rao and Daugh, 1990), such as disruption of gene expression caused by phytohormone (Jones 1991; Paranjothy et.al. 1993), callus structure is in compact calli, nodular calli or crumbs calli with its the rapid growth produces 5 - 10 % and 100 % abnormal plants (Pannetier et al. 1981; Duran et al. 1993).According Ginting and Fatmawati (1996)which their study corresponded with Paranjothy *et al*. (1993), abnormalities have relation with the length and age of callus subculture.Eeuwens *et al*. (2002) suggested that mantled flowers can be ocurred during culture condition in the embryo multiplication. Abnormalities can also occur in male and female flowers that will be developed intoparthenorcarpy fruit or mantled fruit (Corley et al. 1986).

DNA analysis is one of the molecular biologyapproaches to identify a genotype. The main advantage of genotype analysis is DNA level changes with genetic distance between individual with another can be investigated(Serret *et al*. 1997). Recently, the development of a new technology has been able to develop the DNA polymorphism analysis for genetic mapping, MAS, genomic fingerprinting. These technologies include RFLP, RAPD, AFLP and microsatellite (SSR).Randomly Amplified DNA fingerprinting (RAF) is the primary amplification technique based on arbitrary (Waldron et al. 2002)

RAF (Randomly Amplified DNA fingerprinting) is based on the technique of amplification of arbitraryprimers(Waldron *et al*. 2002). RAF technique is similar to the DAF. Advantages of RAF over previous protocols are its robustness and reliability, no requirement for highly-purified DNA template, relativdy requires few steps,the opportunity for sensitive detection via-radio-labeling or fluorescent tagging, more markers being simultaneously detected, and the ability to identify codominant loci (Waldron *et al*. 2002).

Randomly amplified polymorphic DNA (RAPD) technique is one of the most frequently techniques used for molecular method. RAPD technique has been widely used to assist activities of plant breeding, including analysis of genetic variation and similarity in various organisms. The application of molecular markers has been developed to overcome problems such as morphological markers, cytology, histology and biochemistry (Akagi *et al*. 1996; Ayers *et al*. 1997).

Hence, the objective of this research was to study the sequence changes of DNA genome from somatic embryo at several growth stages and their plantlets using RAPD and RAF.

MATERIALS AND METHODS

Plant Material: Normal mother plantand somatic embryo of oil palm clone MK 558 and MK 638 were obtained from Oil Palm Research of Indonesia (PPKS), Medan.

DNA extraction: DNAs for RAPD and RAF analysis were extracted from young leaves of normal mother plant and somatic embryo of oil palm using modified method of Doyle& Doyle (1990).

RAPD Assay: DNA of each sample is used for PCR analysis, respectively by employing 10 primer10-mer with the composition of sequences shown on Table 1. Amplification reaction wascarried outat volume of 25 ul. Composition of PCR analysis is as follows: 5.0 ul DNA template (5 ng/ul) ,0.2 ul dNTP, 2.5 ul PCR buffer (10X) + Mg Cl2, 1.0 ul primer (10 pmalul), 0.2 ul Taq Polymerase (5U/ul), and 16.1 ul ddH2O.The amplification was performed using aThermal Cycler Gene PCR (ABI 9700).The mixture was pre-denaturated at 94°C for 4 min. The PCR conditions were as

follows: 45 cycles at 94°C for 1 min for denaturation, 36°C for 1 min for annealing, 72°C for 2 min for extension and 72°C for 4 min for post-extension.

Amplified products were analyzed by electrophoresis. PCR results could be then fractionated by using agarose gel 1.4% (w/v) in 40 ml 1X TAE solution. Electrophoresis wascarried out at 75 volts for 1.5 h. Results of electrophoresis were documented with Kodak Logic Dott Software.

RAF Assay: The sampleswere somatic embryo and normal mother plant of clone MK 638. The RAF technique was performed according to the general steps as described by Waldron *et al.*, (2002). Each reaction volume of 10 uL cointained 1x PCR buffer (10mM Tris pH 8.0, 10mM KCl, 5 mM MgCl2), 20uM dNTPs, 1,5 units AmpliTaqI Stoffel Fagment DNA polymerase, 1 uCi α-labelled ^{33}P-dATP, and 5 uM single oligonucleotides (Operon Technologies Inc.A kits) and 50 ng of genomic DNA template. PCR was performed with a hot start (85°C), an initial denaturation at 94 °C for 5 min, followed by 30 amplification cycles of : 94 °C for 30s, 60s each at 57 °C,56°C, 55°C,54°C,53°C and final extension step at 72 °C for 5 min. Eight primers were initially tested.

Furthermore, DNA analyzer (3130 DNA Analyser - Applied Biosystems) was used to find DNA fragments. GeneScan™-500LIZwas used as size Standard. As much as 2 ul sample of PCR aliquot result was then used and added with 0,2 ul GeneScan™-500LIZ and 7.8 uL HiDi formamide, denatured at 95°C for 5 min and finally cooled on ice.

Table 1.Primer sequences from Operon Technologies Inc.

No	Primer	Primer sequence (5' – 3')
1.	AB-16	5'-CCCGGATGGT-3'
2.	AE-11	5'-AAGACCGGGA-3'
3.	AO-12	5'-TCCCGGTCTC-3
4.	AP-20	5'-CCCGGATACA-3'
5.	BB-18	5'-CAACCGGTCT-3'
6.	W-15	5'-ACACCGGAAC-3'
7.	OPB-06	5'-TGCTCTGCCC-3'
8.	OPC-02	5'-GTGAGGCCTC-3'
9.	OPC-08	5'-TGGACCGGTG-3'
10.	OPC-09	5'-CTCACCGTCC-3'
11.	OPE-14	5'-TGAGGCTGAG-3'
12.	SC-10-19	5'-CGTCCGTCAG-3'

RESULTS AND DISCUSSION

A. RAPD Assay

The results of RAPD analysis on clone MK 638 and MK 558 produced polymorphic pattern band that could differentiatenormal SE cotyledon and abnormal SE cotyledon. Specifically on clone MK 638, both normal and abnormal SE cotyledon could be distinguished using Primer OPE-14 with DNA fragment were at 3500 bp, 2250 bp, 1750 bp, 1400 bp and 850 bp. Similarity between primer OPE-14, W-15, AP-20 was that they could distinguish either normal SE cotyledon or abnormal Se cotyledon at DNA fragment of 1750 bp. Moreover, Primer SC-10-19 could distinguish normal and abnormal SE globular at DNA fragment of 5000 bp, 4000bp, 3000 bp and 2250 bp.

However, as compared to clone MK 638, on clone MK558 differentiation between normal and abnormal SE cotyledon using primers pre-mentioned above occurred at different DNA fragments. For instance, OPE-14 could distinguish both normal and abnormal SE cotyledon at the DNA fragment f 2500 bp, 3000 bp. Whilst primer AP-20 produced DNA fragment at 3000bp, 2000bp and 1750 bp and primer W-15 produced DNA fragment DNA at 5000 bp and 2500 bp (Table 1 and Figure 1 and 2). Consequently, using primers OPE-14, OPC-09, AP-20 and SG-10-19 could producepolymorphic fragments of DNA for either plantlet or mother plantleaves on clone MK 638 and MK 558.

According to Grattapaglia *et al.*,(1992), the numberof polymorphic DNAbands shows the state of the plant genome whereas the differences in the number and polymorphic fragment DNA generated by every primer show the complexity of plant genomes.Moreover, Phillips *et al.* (1990) suggested plants regenerated from callus and relatively non-differentiated causes the possibility of a large number ofgenetic changes. This study corresponds with those, which could be explained from the results in which Primer OPE-14 and SC-10-19 produced mostly polymorphic bands on the SE cotyledon.

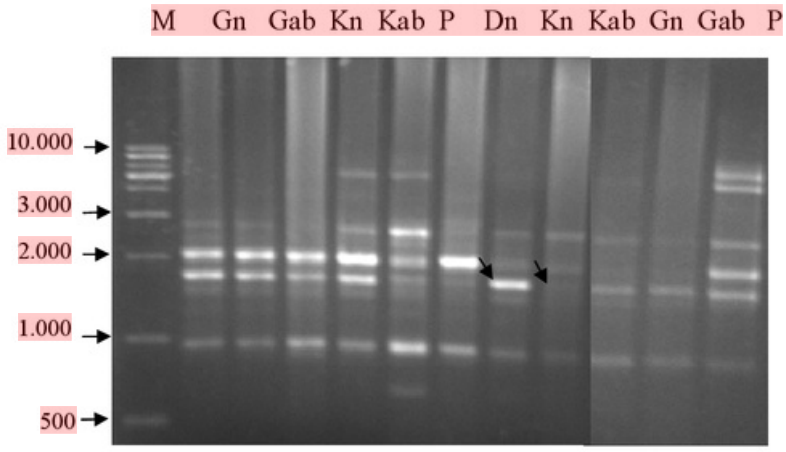

Fig. 1. Primer W-18. M (marker 1kb), Clone 558 (lane 2-7) ; Normal globular somatic embrio (Gn), Abnormal Globular (Gab), Normal cotyledon (Kn), Abnormal cotyledon (Kab), Planlet (P), Leaves of mother plant (Dn). Clone 638 (lane 8 - 12) ; Normal cotyledon somatic embryo (Kn), Abnormal cotyledon (Kab), Normal Globular (Gn), Abnormal Globular (Gab), Planlet (P).

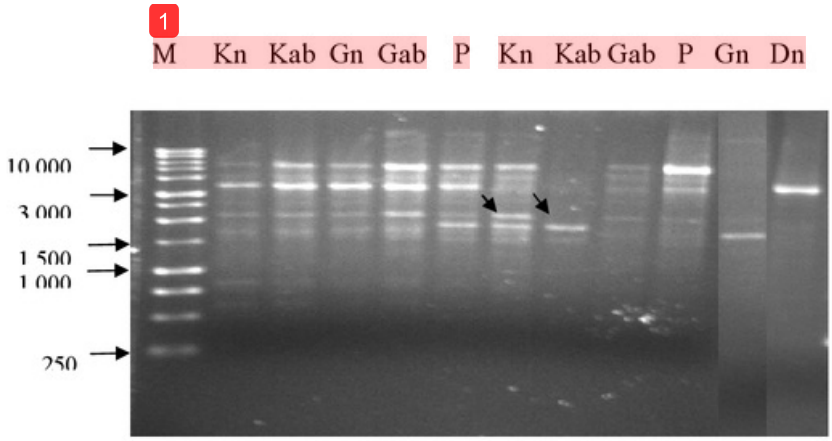

Figure 2. Primer of SC 10-19. M (marker 1kb), Clone 558 (lane 2-6) ; Normal globular somatic embrio (Gn), Abnormal Globular (Gab), Normal cotyledon (Kn), Abnormal cotyledon (Kab), Planlet (P). Clone 638 (Lane 7 -12) ; Normal cotyledon somatic embryo (Kn), Abnormal cotyledon (Kab), Abnormal SE Globular (Gn), Planlet (P), Normal SE globular and Leaves of mother plant (Dn).

Jones (1991) and Paranjothy *et al*. (1993) statedthe abnormality is occurredon oil palm clonethat caused by gene expression changes. Abnormality is found on flowering of oil palm clone that is usually caused by2.4- D as a plant growth regulator. The aim of

addition of 2,4 -Dinto the media is to induce callus and embryonic formation and has been reported by Gintinget *al.*(1991) at Marihat Research Institute that indicates the propagation of oil palm clones with addition of 2.4 D.Aside from addition of 2,4 -Dinto the media, Edwin (1993) reportedsomaclonal variation causedbyabnormalities could also occur due to utilization of mercury chloride as solvent/solution for the explants sterilization.High concentration of mercury chloride ould cause amino acid compositions of plant tissue change and resulting in disruption of protein synthesis.

Tabel 1. Fragment polymorphisms by RAPD technique onMK638 dan MK 558 clone.

DNA fragment pb	Clone 638						Clone 558					
	OPE14 (5'-TGCGGCTGAG-3')						OPE14 (5'-TGCGGCTGAG-3')					
	G+	G-	K+	K-	D	DN	G+	G-	K+	K-	D	DN
1. 3500	+	+	+-	+	+		+	+	-	+	+	+
2. 3000)	+	+	+	+	+	+	+	+	-	+	+	+
3. -	+	+	+	+	+	+	+	+	+	+	+	+
4. 2250	+	+	+	-	+	+	+	+	+	+	+	+
5. 1750	+	+	+	-	+	-	+	+	+	+	+	-
6. 1400	-	-	-	+	-	+	-	-	-	-	-	+
7.	+	+	+	+	+	+	+	+	+	+	+	+
8. 850	-	-	-	+	-	-	-	-	-	-	-	-
9. 750	-	-	-	-	-	+	-	-	-	-	-	-
DNA fragmentpb	W-15 (5'-ACACCGGAAC-3')						W-15 (5'-ACACCGGAAC-3')					
	G+	G-	K+	K-	D	DN	G+	G-	K+	K-	D	DN
1. 5000	+	+	-	-	+	-	+	+	-	+	+	-
2. 2500	-	-	-	-	+	-	+	+	-	+	+	-
3.	+	+	+	+	+	-	+	+	+	+	+	-
4.	-	-	-	-	-	+	+	+	+	+	+	+

5.	-	-	+	+	+	-	+	+	+	+	+	-	
6. 1750	-	-	+	-	-	-	+	+	+	+	+	-	
7. 1500	+	+	+	-	+	-	+	+	+	+	+	-	
8.	-	-	-	-	-	+	-	-	-	-	+	+	
9.	-	-	+	+	-	+	-	-	-	-	-	+	
10.	+	+	-	-	+	-	-	-	-	-	-	-	
DNA fragment pb	OPC-09(5'CTCAACGTCC-3')						OPC-09(5'CTCAACGTCC-3')						
	G+	G-	K+	K-	D	DN	G+	G-	K+	K-	D	DN	
1.3250	-	-	+	-	-	-	+	+	+	+	+	-	
2.	+	+	+	+	-	-	+	+	+	+	+	-	
3.3000	+	+	+	+	+	+	+	+	+	+	+	+	
4. 2000	-	-	+	-	-	+	+	+	+	+	+	+	
5.	+	+	+	+	-	-	+	+	+	+	+	+	
6.	-	-	-	-	-	-	-	-	-	-	-	+	
7.	+	+	+	+	-	-	+	+	+	+	+	-	
8.	-	-	-	-	-	-	-	-	-	-	-	+	
DNA fragmentpb	AP-20(5'-CCCGGATACA-3')						AP-20(5'-CCCGGATACA-3')						
	G+	G-	K+	K-	D	DN	G+	G-	K+	K-	D	DN	
1. 3000	-	-	- +	-	-		-	-	+	-	-	-	
2. 2000	-	-	-+	-	-		-	-	+	-	-	-	
3. 1750	-	-	-+	-	-		-	-	+ -	-	-		
4.	+	+	+	+	+	+	+	+	- +	+	+		
5.	+	+	+	+	+	+	+	+	-	+	+	+	
6. 750	+	+	+	+	+	-	+	+	+	+	+	-	
7.	+	+	+	+	+	+	+	+	+	+	+	+	
DNA fragmentpb	SC10-19 (5'-SGTCCGTCAG-3')						SC10-19 (5'-SGTCCGTCAG-3')						
	G+	G-	K+	K-	D	DN	G+	G-	K+	K-	D	DN	

1. 5000	-		+		+		-+		-			+		+		+		+		+		-	
2. 4000	-		+		+		-+		-			+		+		+		+		+		-	
3. 3000	-		+		+		-		+		+	+		+		+		+		+		+	
4. 2250	-		+		+		-+		-			+		+		+		+		+		-	
5. 2000	+		-		+		+		-		-	+		+		+		+		+		-	
6.	-		-		+		+		-		-	+		+		+		+		+		-	

Note : Band (+), No band (-), Normal Globular (G+), Abnormal Globular (G-), Normal Cotyledon (K+), Cotyledon(K-), Plantlet leaves (D), Motherplant leaves (DN).

B. RAF Assay

Through RAF analysis, there were six DNA genomes were amplified using primers. Those could be amplified by Primer AB-16 (5'-CCCGGATGGT-3 '), whereas using primer AO12 (5'-TCCCGGTCTC-3') produced at least one fragment DNA. Furthermore, most polymorphic fragments could be amplified with primer AP-20 (5'-CCCGGATACA-3') (Picture 3 dan Tabel 2).

Normal and abnormal SE cotyledoncould be distinguished due to a change in sequence of DNA genome. DNA genome of normal and abnormal SE cotyledon, plantletleaves and mother plantleaveswere amplified using primers AB-16, AE-11, AP-20, AO-12, BB-18 and W-15. SE cotyledon could produce 1-7 fragments from each primer with total of 24 fragments and size of 90 -358 bp. Changes in the DNA sequences of normal and abnormal cotyledon SE could be detected at approximately 90 bp, 91bp and 146 bp using Primer AB-16. The primer AO-12 produced one polymorphic fragment at 150 bp, while others, such as primer AE-11, AP-20, BB-18 and W-15 produced two polymorphic fragments(Table 2). Moreover, the Primer AO-12, W-15 and BB-20 could distinguish both normal and abnormal SE cotyledons at a specific fragment of about 150 bp (Table 2).

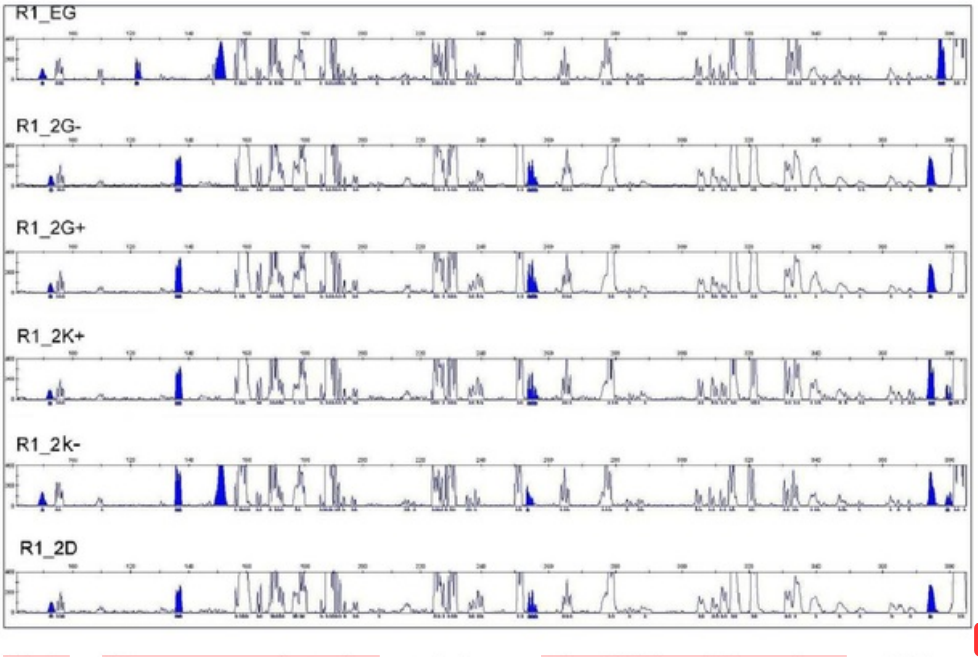

Fig.3. Fluorecence detection technique of RAF with primer AB6 (5'-CCCGGATGGT-3').Labeled by FAM (6-carboxy-fluorecein) on oil palm plant clone 638. The normal mother plant DNA genome (EG), Normal SE globular (2G+), Abnormal SE globular (2G-),Normal SE cotyledon (2K+), Abnormal SE cotyledon (2K-) and Plantlet leaves (2D).

Form the results, both RAF and RAPD techniquesemployed the same primers (W-15 and AP-20) to produce polymorphic amplification. The results of RAF analysis could be detected at around 100-400 bp and until at level of single-nucleotide base difference using electrograph (Fig.3), whilst RAPD analysis produced polymorphic bands at 1500-5000 bp (Fig. 1 and 2). The results of RAPD and RAF showed a change in normal and abnormal SE cotyledon of plantlet and mother plantleaves (Fig 1 and 2). The Results RAF using primers W-15 produced a change in DNA sequence at 150 bp, whereas the RAPD analysis using primer W-15 produced a change DNA sequence at 1750 bp (Table 1 and 2).The RAF technique could detect DNA sequence changes until 1-10bp.Thechanges of DNA sequence that could detect the location until one base called mutation. Conclusively, the morphology change of normal SE cotyledon became

abnormal cotyledon could possibly be caused by changes in DNA sequence at specific sites (Table 2).

Table 2. Fragment polymorphisms by RAF technique on MK638 clone

Fragmen DNA (pb)	AB- 16 (5'-CCCGkasiGATGGT-3')						AO-12 (5'-TCCCGGTCTC-3')					
	2K+	2K-	2G+	2G-	2D	DN		2K+	2K-	2G+	2G-	2D DN
90	-	+	-	-	-	+	150	-	+	-	-	-
91	+	-	+	+	+	-	160	+				
138	+	+	+	+	+	-		+	-	+	+	+
146	-	+	-	-	-	+		-				
248	+	+	+	+	+	-						
258	+	+	+	+	+	-						
375	+	+	+	+	+	-						
378	-	-	-	-	-	+						
380	+	+	-	-	-	-						
Fragmen DNA (pb)	AE-11(5'-AAGACCGGGA-3')						BB-18	(5'-CAACCGGTCT-3')				
	2K+	2K-	2G+	2G-	2D	DN		2K+	2K-	2G+	2G-	2D DN
152	-	+	-	-	-	+	90	-	+	-	-	- +
158	+	-	+	+	+	-	150	-	+	-	-	- +
369	-	-	-	-	+	-	158	+	-	+	+	+ -
372	+	+	+	+	+	+						
Fragmen DNA (pb)	AP-20 (5'-CCCGGATACA-3')						W-15(5'-ACACCGGAAC-3')					
	2K+	2K-	2G+	2G-	2D	DN		2K+	2K-	2G+	2G-	2D DN

115	-	+	-	-	-	-	150	-	+	-	-	-
155	-	+	-	-	-	+	214	+				
238	+	+	+	+	+	-	217	+	+	+	+	+
355	+	-	+	+	+	-	255	-				
358	+	-	+	+	+	-	258	+	+	+	+	+
								-				
								-	-	-	-	-
								+				
								-	-	-	-	-
								+				

Keterangan : Fragment (+), Fragment (-), Normal Globular (G+), Abnormal Globular (G-), Normal Cotiledon (K+), Abnormal Cotiledon (K-), Planlet Leaves (D), Mother plant leaves (DN).

CONCLUSION

In conclusion that RAPD technique showed similarity between primer OPE-14, W-15, AP-20 was that they could distinguish either normal SE cotyledon or abnormal SE cotyledon at DNA fragment of 1750 bp on clone MK638. The RAF technique detected the changes of DNA genome at 90 – 358 bp. The Primer AO-12, W-15 and BB-20 could distinguish both normal and abnormal SE cotyledons at a specific fragment of about 150 bp by using RAF technique.There were three amongstsix primers (AO-12, BB-18, W-15) genomic DNA sequences were detected at 150 bp. Consequently, RAPD and RAF technique with the primers W 15 and AP 20 could distinguish normal and abnormal SE cotyledon, plantlet and normal mother plant.

ACKNOWLEDGMENTS

The authors would like to thank the Oil Palm Research Center of Indonesia (PPKS) for providing somatic embryo and mother plant samples. We also would like to thank Dr. Rudy Lukman for his permission to work with RAF technique in the laboratory of the Biotechnology Department at PT. BISI International Tbk.

REFERENCES

Akagi H, Yokozeki Y, Inagaki A, Fujimura T. 1996. Microsatellite DNA markers for rice chromosomes. *Theor.Appl.Genet.* 93 : 1071-1077.

Ayers NM *et al.* 1997. Microsatellites and single – nukleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germ plasm. *Theor Appl. Genet.* 94 : 775 -780.

Corley RHV, Lee CH, Law LH, Wong CY. 1986. Abnormal flower development in oil palm clones. *Planter* (Kuala lumpur) 62 : 233-240.

Deambrogio E, Dale PI. 1980. Effect of 2,4 D on the frequency of regenerated plant in barley (*Hordeum vulgare*) cultivar 'Akka' and on genetic variability between Them. Cereal Res. *Commun* 8 : 417 - 424.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fersh leaf tissue. *Phytochem Bull.* 19 : 11 -15.

Duncan RR. 1997. Tissue culture-induced variation and crop improvement. *Adv. Agron.* 58 : 201- 240.

Edwin FG. 1993. Plant Propagation by Tissue Culture. Part 1: 2nd Edition. Exegetics Ltd. Edington, Wilts-England. p.420-425.

Ginting G, Fatmawati. 1997. Suspensi sel pada kelapa sawit (*Elaeis guineensis* Jacq.). *J. Pen. Kelapa Sawit* 5 (3) : 153 -160.

Ginting G, Lubis RA, AU Lubis. 1991. Kultur jaringan kelapa sawit (*Elaeis guineensis*) di Pusat Penelitian Perkebunan Marihat. Seminar Bioteknologi Perkebunan dan Lokakarya Biopolimer PAU-IPB, Bogor. P.115-119.

Grattapaglia D *et al.* 1992. Mapping in Woody Plant with RAPD Marker: Application to Breeding in Forestry nd horticulture. P 37 -40. In : Aplication of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series CSSA/ASHS/AGA, Minneapolis, MN.

Grifftihs AJF, Suzuki J, Miller H, Lewontin RC. 1993. An Introduction to genetic Analysis. Fifth edition. W.H. freeman and Co. 840p.

Jones LH. 1991. Endogenous cytokinin in oil palm (*Elaeis guineensis* Jacq.) callus, embryoids and regenerant plants measured by radioimmunoassay. *Plant Cell Tiss. Org. Cult* 20 : 201 - 209.

Kaeppler SM, Kaeppler HF, Rhee Y. 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Mol Biol* 43 : 179 -188.

Kumar A. 1995. Somaklonal variation and molecular genetics department. Scottish Crop Research Institute, Invergrowrie Dundee. Canada. p. 197-212.

Larkin PJ, Scowcroft WR. 1981. Somaclonal variation-a novel source of variability from cell culture for plant improvement. *Theor.Appl.gen.* 60 : 197 -214.

Paranjothy K, Othman R, Tan CC, Wang G, Soh AC.1993. Incidence of abnormalities in relation to in vitro protocols. *Di dalam* : Rao V. *et al*, editor. *Recent Devin Oil Palm Tissue Cult & Biotechnol*. Kuala Lumpur, p. 131 -141.

Pannetier C, Arthius P, LievouxDE, 1981. Neoformation de jeuneus planlets d'*Elaeis guineensis* a partier de eals primaries obttenus sur fragments foliaires in vitro. *Oleagineux* 36: 119 - 122.

Phillips RL, Plunkett DJ, Kaeppler SM. 1990. Do we understand somaclonal variation? Di dalam : H.J.J. Nijkamp *et al*. editor. Progress in Plant Cellular and Molecular Biology. Proc. 7th Internat. Cong. Plant Tiss. *Cell Cult*., p. 131 – 14.

Serret ND, Udupa SM, and Weigand F. 1997. Assement of genetic diversity of cultivated chickpea using microsatellite-derivate RFLP markers implication for origin. *Plant Breed*. 116 : 573-578.

Van Harten AM. 1998. Mutation Breeding. Theory and Practikal Applications. CambribgeUniv. Press. Wong, G., S.P. Chong, C.C tan, and A.C Soh. 1999. Liquid Suspension culture-A potential technique for mass production of oil palm clones. Palm oil Res. Inst. Of Malaysia. P: 3-10.

Waldron J, *at al*. 2002. Randomly amplified DNA fingerprinting : a culmination of DNA marker technologies based on arbitrarily-primed PCR amplifcation. *J Biomed and Biotech* 2 (3) : 141-150.

(Check) 11 - abnormality_analysis_of_oil_palm

ORIGINALITY REPORT

%94	%94	%6	%1
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.unpad.ac.id Internet Source	%94

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		